Pacific Science (1982), vol. 36, no. 2


# Inheritance of Rugose Leaf in *Desmodium*[1]

K. H. Chow[2]

ABSTRACT: Four plants with rugose leaflets were found among 19 interspecific double-cross hybrid plants of *Desmodium*. It was found through test crosses and progeny tests that rugose leaflet was controlled by three pairs of genes which were complementary in action, with each of the three *Desmodium* species contributing one dominant gene.

THE GENUS *Desmodium* belongs to the subtribe Desmodiinae of the tribe Coronilleae. It is distributed mainly in the tropics and subtropics of the world, and comprises 350–450 species, showing a remarkable diversity in various morphological characters (Ohashi 1973). Two important pasture legumes in Hawaii are *D. intortum* (Mill.) Urb. (cv. greenleaf) and *D. uncinatum* (Jacq.) D.C. (cv. silverleaf) (Takahashi 1952, 1956, Younge, Plucknett, and Rotar 1964). These species are also important in the wetter coastal areas of Queensland, Australia, where seeds are commercially available (Bryan 1966, 1969, Hutton 1968). *Desmodium sandwicense* E. Mey. has also been tried in Hawaii with varying degrees of success (Rotar and Chow 1971).

Hutton (1960) reported that these three *Desmodium* species were cross-pollinated but that they also set selfed seeds readily. Hutton and Gray (1967) hybridized the three species, and noted that $F_1$ progenies could be identified by the longer hairs of *D. uncinatum*, brown flecks on *D. intortum*'s leaflets, as well as the silver marking coming from the leaflet midrib of *D. sandwicense* and *D. uncinatum*. Chow (1971) and Chow and Crowder (1974*a*) reported the photoperiodic requirements, breeding behavior, and hybridization of these three species. In addition to morphological characteristics, isozyme patterns determined by zone electrophoresis were used by several workers to identify true hybrids (Chow 1975, Chow and Crowder 1974*b*, 1975). McWhirter (1969) reported cytoplasmic male sterility in *Desmodium* and presented a scheme utilizing the male sterility for commercial production of the interspecific hybrid *D. sandwicense* × *D. intortum* as a cultivar.

In an interspecific breeding program, 4 plants with rugose leaves were found among 19 double-cross hybrid plants. The rugose leaves curve backward, and are strikingly different from the normal flat *Desmodium* leaves (Figure 1). This characteristic is caused by the shortening of the midrib and main veins of leaves. Chow (1968) first found this character in *Desmodium* in Hawaii and reported that the plants flowered from November to April, the same flowering period as *D. uncinatum*. The percentage of shrivelled seeds was only 5.0–9.5 as compared with 13.46 for the three-species hybrids. The 1000-seed weights of the rugose-leaved plants were 3.73–3.76 g, about midway between those of *D. uncinatum* and *D. sandwicense*, and were much heavier than those of *D. intortum*, which were less than 2 g. The internodes of rugose plants were short and varied from 3.1 to 5.5 cm, the stems were densely covered with long, hooked hairs, and stem color varied from green to red. Leaflets were medium in size, with leaflet size indices (leaflet length × leaflet width) ranging from 12 to 16; leaf hairs were dense and long; and leaf color varied from brown to green. The overall appearance of the plants resembled each of the three parental species to a certain extent, except for the rugose-leaf characteristic which was never observed in any of the three species by itself or in any $F_1$ hybrids among the three species. The objectives of this work were to study the inheritance of this

[1] Manuscript accepted 12 January 1982.
[2] National University of Singapore, Department of Botany, Faculty of Science, Lower Kent Ridge Road, Singapore 0511.

FIGURE 1. Rugose leaves and raceme of a rugose-leaved plant.

striking leaf characteristic through $F_2$, $F_3$, and back-cross populations.

MATERIALS AND METHODS

In an interspecific hybridization program in the genus *Desmodium* performed in Hawaii, 4 plants with rugose leaflets were found among 19 double-cross hybrid plants with the combination of (*D. sandwicense* ♀ × *D. intortum* ♂) ♀ × (*D. uncinatum* ♀ × *D. intortum* ♂) ♂. Hybrid $F_2$ seeds were harvested from the four rugose-leaf plants and germinated in petri dishes. Germinated seeds were transplanted into 20-cm pots containing garden soil. The pots were kept in the garden of the Department of Botany at National University of Singapore (Bukit Timah Campus) under natural sunlight. The plants were watered daily and supplied with fertilizer (Welgrow) every week. The $F_2$ plants with rugose leaves were back-crossed to the parents by the following method: Flower buds were emasculated in late afternoon by opening the end of the keel petals with fine-pointed forceps and removing the ten anthers. Racemes bearing the emasculated flower buds were covered with maize pollination bags (No. 317). Pollinations were carried out the next morning, around 9 AM. Pollen was collected by toothpicks and transferred to the stigmas of the female plants. Pollinated flowers were covered again with pollination bags. Wet cotton balls were put in the bags to increase the humidity and raise the percentage of seed set. The bags were removed from the raceme 3 days after pollination.

The $F_2$ plants were selfed to provide $F_3$ seeds by covering racemes with bags, as before. The bags were removed after 10 days.

Seeds obtained from back-crosses and self-pollination were dried at 40°C for 2 days and then scarified by rubbing between sandpaper before germination test on wet tissue paper placed in petri dishes. Germinated seeds were transplanted in vermiculite placed in flats 45 cm × 30 cm. The flats were each sown with 25 germinated seeds, and were kept in the garden under natural sunlight and watered and fertilized as necessary. The rugose-leaf characteristic can easily be identified at the seedling stage. After 5 weeks, the seedlings were examined for leaf characteristics and counted. A chi-square test was employed to analyze the data.

## RESULTS AND DISCUSSION

Seeds harvested from rugose-leaf plants grown in Hawaii in 1968 were germinated in petri dishes in 1975. After 7 yr of storage, seed germination averaged only 60 percent. Most seeds with hard seed coats were still viable and germinated after 7 yr of storage while all those with soft seed coats failed to germinate.

Segregation of leaf characteristic occurred in the $F_2$ generation (Table 1). The $F_2$ plants segregated into roughly 1:1, 1:2, and 1:3 ratios for rugose leaf to flat leaf. Due to the small numbers of $F_2$ plants and uncontrolled pollination, the results were not conclusive; thus no chi-square test was made for the data.

Three $F_2$ plants with rugose leaves were back-crossed to their parents. They were chosen because of their profuse flowers. They were used as female parent and crossed with *Desmodium sandwicense* and *D. uncinatum*. *Desmodium intortum* was not entered for back-cross study because it did not flower under the natural day length of Singapore (Chow 1974). Wong (1961) reported that this species is a short-day plant and flowered only during the cooler season in Taiwan.

The results of back-crosses are given in Table 2. The average success of back-crosses was about 9 percent. Rotar and Chow (1971) reported that the success of interspecific crosses among *Desmodium* species ranged from 2.3 to 15.3 percent, depending upon the species and strains. Chow and Crowder (1973) obtained 21.5 percent success by the tripping method, but only 3.5 percent success by an emasculation method in a hybridization study of *Desmodium* species. However, the tripping method resulted in a high amount of selfing and thus was not recommended.

When *Desmodium sandwicense* and *D. uncinatum* were used as the male parent and back-crossed to the rugose-leaf $F_2$ plants, the former had less success, about 7.3 percent, than the latter, about 11 percent. The rugose-leaf plants resembled *D. uncinatum* more than they did *D. sandwicense* in morphology and flowering behavior. *Desmodium* plants with a higher degree of morphological resemblance were more cross-compatible.

Seeds obtained from back-crosses were scarified and germinated in petri dishes. Seed germination averaged 83 percent. This was statistically significantly lower than the germination percentage of open-pollinated seeds harvested from the three *Desmodium* species, which is usually higher than 95 percent. Germinated seeds were transplanted in vermiculite in flats 45 cm × 30 cm. Five-week-old seedlings were examined for rugose leaves and counted. The data are shown in Table 3.

Rugose-leaf $F_2$ plant #1 showed no segregation when crossed with *Desmodium sandwicense*, but showed a 1:1 ratio when crossed with *D. uncinatum*. Rugose-leaf $F_2$ plant #2 showed a segregation ratio of 1:1 when crossed with both *D. sandwicense* and *D. uncinatum*. Rugose-leaf $F_2$ plant #3 showed a 1:1 segregation ratio when crossed with *D. sandwicense*, but did not show any segregation when crossed with *D. uncinatum*. All calculated chi-square values were smaller than 2.71, which is the theoretical chi-square value at 0.10 probability of a greater value. However, the observed frequencies of rugose-leaf plants were consistently higher than the expected frequencies. This was probably due to selfing, which might occur in back-crosses. It is concluded from the back-cross results that rugose leaf was controlled by at least two pairs of genes, and rugose-leaf $F_2$ plant #1 and plant #3 were homozygous at one locus and heterozygous at the other, while plant #2 was heterozygous for at least two loci. However, the genotypes of the rugose-leaf plants could not be determined from the back-cross results.

To work out the genotypes of the rugose-leaf plants, five rugose-leaf $F_2$ plants were selfed for $F_3$. One hundred $F_3$ seeds from each of the five rugose-leaf $F_2$ plants were sown in flats, and the $F_3$ seedlings at 5 weeks were

TABLE 1

PERCENTAGE OF SEED GERMINATION AND SEGREGATION OF RUGOSE LEAF IN $F_2$ GENERATION

| RUGOSE-LEAF $F_1$ PLANTS | NUMBER OF SEEDS TESTED | NUMBER OF SEEDS GERMINATED | PERCENT SEED GERMINATION | NUMBER OF PLANTS ESTABLISHED | SEGREGATION IN $F_2$ | |
|---|---|---|---|---|---|---|
| | | | | | RUGOSE | FLAT |
| Plot no. 1217 | 32 | 20 | 63 | 16 | 8 | 8 |
| Plot no. 1318 | 35 | 23 | 66 | 21 | 11 | 10 |
| Plot no. 2020 | 28 | 15 | 54 | 15 | 4 | 11 |
| Plot no. 2812 | 29 | 17 | 59 | 12 | 4 | 8 |
| Totals or average | 124 | 75 | 60 | 64 | 27 | 37 |

TABLE 2

POD FORMATION, SEED SET, SEED GERMINATION, AND PLANT ESTABLISHMENT OF BACK-CROSSES

| BACK-CROSSES | CROSSES MADE | PODS FORMED | PERCENT POD FORMATION | SEEDS HARVESTED | SEEDS PER POD | SEEDS GERMINATED | PERCENT SEED GERMINATION | SEEDLINGS ESTABLISHED |
|---|---|---|---|---|---|---|---|---|
| 1. Rugose-leaf $F_2$ #1 × *D. sandwicense* | 125 | 9 | 7.2 | 36 | 4.0 | 29 | 80.5 | 27 |
| 2. Rugose-leaf $F_2$ #1 × *D. uncinatum* | 131 | 12 | 9.2 | 50 | 4.2 | 42 | 84.0 | 40 |
| 3. Rugose-leaf $F_2$ #2 × *D. sandwicense* | 127 | 9 | 7.1 | 43 | 4.8 | 38 | 88.4 | 33 |
| 4. Rugose-leaf $F_2$ #2 × *D. uncinatum* | 88 | 11 | 12.5 | 44 | 4.0 | 36 | 81.8 | 36 |
| 5. Rugose-leaf $F_2$ #3 × *D. sandwicense* | 120 | 9 | 7.5 | 34 | 3.8 | 28 | 82.4 | 26 |
| 6. Rugose-leaf $F_2$ #3 × *D. uncinatum* | 106 | 12 | 11.3 | 46 | 3.8 | 37 | 80.4 | 30 |
| Totals or average | 697 | 62 | 8.9 | 253 | 4.08 | 210 | 83.0 | 192 |

TABLE 3

SEGREGATION RATIOS OF RUGOSE LEAF IN BACK-CROSS POPULATIONS AND CHI-SQUARE TEST DATA

| BACK-CROSSES | OBSERVED FREQUENCIES RUGOSE | OBSERVED FREQUENCIES FLAT | EXPECTED FREQUENCIES* RUGOSE | EXPECTED FREQUENCIES* FLAT | CHI-SQUARE | PROBABILITY OF A GREATER VALUE |
|---|---|---|---|---|---|---|
| 1. Rugose-leaf $F_2$ #1 × *D. sandwicense* | 27 | 0 | — | — | — | — |
| 2. Rugose-leaf $F_2$ #1 × *D. uncinatum* | 24 | 16 | 20.0 | 20 | 1.60 | 0.10–0.25 |
| 3. Rugose-leaf $F_2$ #2 × *D. sandwicense* | 19 | 14 | 16.5 | 16.5 | 0.76 | 0.25–0.50 |
| 4. Rugose-leaf $F_2$ #2 × *D. uncinatum* | 21 | 15 | 18.0 | 18.0 | 1.00 | 0.25–0.50 |
| 5. Rugose-leaf $F_2$ #3 × *D. sandwicense* | 16 | 10 | 13.0 | 13.0 | 1.38 | 0.10–0.25 |
| 6. Rugose-leaf $F_2$ #3 × *D. uncinatum* | 30 | 0 | — | — | — | — |

*Expected frequencies are calculated based upon 1 : 1 ratio.

classified into rugose-leaf and flat-leaf plants and counted. The results are given in Table 4.

The $F_3$ seedlings from the five rugose-leaf $F_2$ plants showed different segregation ratios. Chow (1968) reported that rugose leaf in *Desmodium* was caused by the shortening of the leaf midrib and main veins. This characteristic was not found among the parental species and single-cross hybrids. It was only found in the double-cross hybrids involving three *Desmodium* species. This indicates that the shortening of midrib and main veins is elaborated owing to interaction of the dominant alleles of several genes from the three *Desmodium* species. Furthermore, the action of these dominant alleles is complementary, i.e., each allele controls a step essential for the shortening of leaf midrib and main veins. The absence of any one of them throws the whole shortening mechanism of leaf midrib and main veins in the plant out of order. Since there were three *Desmodium* species entered in the double-cross study, it was assumed that rugose leaf was controlled by three complementary genes.

To test the above assumption, chi-square values were calculated based on 3:1 for one pair of genes, 9:7 for two pairs of complementary genes, and 27:37 for three pairs of complementary genes. The $F_3$ seedlings of rugose-leaf $F_2$ plant #2 segregated into 26 and 45 for rugose and flat, respectively, which is not significantly different from 27:37 for three pairs of genes (chi-square = 0.92, df = 1, $p =$ 0.25 – 0.50). It may therefore be concluded that the rugose-leaf characteristic is controlled by at least, or most likely, three pairs of genes with complementary gene effect. Plants with rugose leaves have the genotype $R_iR_sR_u$, with $R_i$ from *D. intortum*, $R_s$ from *D. sandwicense*, and $R_u$ from *D. uncinatum*. The rugose-leaf $F_2$ plant #2 was heterozygous at all three loci; thus, its genotype was $R_ir_iR_sr_sR_ur_u$. Results from back-crosses also indicate that this plant is heterozygous for at least two loci.

The selfed seedlings from rugose-leaf $F_2$ plant #1 segregated into 46 and 30 for rugose and flat, respectively, which is not significantly different from a 9:7 ratio for two pairs of complementary genes (chi-square = 0.57, df = 1, $p$ = 0.25 – 0.50). From back-cross result, it was understood that this $F_2$ plant was homozygous at $R_s$ locus. Therefore, the genotype of this plant must be $R_ir_iR_sR_sR_ur_u$. Similar results were obtained from the selfed progeny of rugose-leaf $F_2$ plant #3. They indicate that this $F_2$ plant is also heterozygous at two loci. Back-cross results indicate that this plant was homozygous at $R_u$ locus. The genotype of this plant was therefore $R_ir_iR_sr_sR_uR_u$.

Selfed progeny of the fourth $F_2$ plant with rugose leaves segregated into 32 and 34 for

TABLE 4

SEGREGATION RATIOS OF RUGOSE LEAF IN $F_3$ GENERATION AND CHI-SQUARE TEST DATA

| RUGOSE-LEAF $F_2$ PLANTS | $F_3$ SEEDLINGS ESTABLISHED | OBSERVED FREQUENCIES | | EXPECTED FREQUENCIES* | | CHI-SQUARE | PROBABILITY OF A GREATER VALUE | CONCLUSION |
|---|---|---|---|---|---|---|---|---|
| | | RUGOSE | FLAT | RUGOSE | FLAT | | | |
| #1 | 76 | 46 | 30 | A. 57.00 | 19.00 | 8.49 | <0.005 | Rejected |
| | | | | B. 42.75 | 33.25 | 0.57 | 0.25–0.50 | Accepted |
| | | | | C. 32.06 | 43.94 | 10.48 | <0.005 | Rejected |
| #2 | 71 | 26 | 45 | A. 53.25 | 17.75 | 55.77 | <0.005 | Rejected |
| | | | | B. 39.94 | 31.06 | 11.13 | <0.005 | Rejected |
| | | | | C. 29.95 | 41.05 | 0.90 | 0.25–0.50 | Accepted |
| #3 | 68 | 43 | 25 | A. 51.00 | 17.00 | 5.02 | <0.025 | Rejected |
| | | | | B. 38.25 | 29.75 | 1.35 | 0.10–0.25 | Accepted |
| | | | | C. 28.69 | 39.31 | 12.35 | <0.005 | Rejected |
| #4 | 66 | 32 | 34 | A. 49.50 | 16.50 | 24.75 | <0.005 | Rejected |
| | | | | B. 37.13 | 28.87 | 1.62 | 0.10–0.25 | Accepted |
| | | | | C. 27.84 | 38.16 | 1.07 | 0.25–0.50 | Accepted |
| #5 | 59 | 41 | 18 | A. 44.25 | 14.75 | 0.96 | 0.25–0.50 | Accepted |
| | | | | B. 33.19 | 25.81 | 4.20 | <0.05 | Rejected |
| | | | | C. 24.89 | 34.11 | 18.03 | <0.005 | Rejected |

*A, calculated based on 3 : 1 ratio for one pair of genes; B, calculated based on 9 : 7 ratio for two pairs of genes; C, calculated based on 27 : 37 ratio for three pairs of genes.

rugose and flat, respectively. Calculated chi-square values were small and acceptable for both 9:7 and 27:37 ratios; thus, this $F_2$ plant could be heterozygous at either two loci or three loci. It had four possible genotypes:

$R_ir_iR_sr_sR_ur_u$ Heterozygous at three loci

$\left.\begin{array}{l} R_iR_iR_sr_sR_ur_u \\ R_ir_iR_sR_sR_ur_u \\ R_ir_iR_sr_sR_uR_u \end{array}\right\}$ Heterozygous at two loci

The exact genotype of this plant can be worked out by growing a larger number of selfed progeny.

The $F_3$ seedlings of the rugose-leaf $F_2$ plant #5 showed a segregation of 41 and 18 for rugose and flat, respectively, which is close to a 3:1 ratio as indicated by the small chi-square value. Therefore, it may be concluded that this plant was heterozygous at one locus, and had three possible genotypes:

$R_ir_iR_sR_sR_uR_u$
$R_iR_iR_sr_sR_uR_u$
$R_iR_iR_sR_sR_ur_u$

The exact genotype of this plant can be determined through back-crosses.

From the results presented above, the assumption of three pairs of genes controlling the rugose-leaf characteristic was established. From the segregation in $F_3$ populations, it was understood that the three genes were complementary in effect. Since $F_3$ plants also showed independent assortment, the three genes were located on three different chromosomes. Pritchard and Gould (1964) and Rotar and Urata (1967) reported that the three *Desmodium* species are diploid, with $2n = 22$.

Chow (1968) reported that 4 plants with rugose leaflets were found among 19 double-cross hybrid plants with the combination of (*D. sandwicense* ♀ × *D. intortum* ♂) ♀ × (*D. uncinatum* ♀ × *D. intortum* ♂) ♂. The genotypes of the three species with regard to rugose leaf were:

$R_iR_ir_sr_sr_ur_u$ for *D. intortum*
$r_ir_iR_sR_sr_ur_u$ for *D. sandwicense*
$r_ir_ir_sr_sR_uR_u$ for *D. uncinatum*

Crosses to obtain rugose-leaf plants are outlined as follows:

*D. sandwicense* ♀ × *D. intortum* ♂
($r_ir_iR_sR_sr_ur_u$) ($R_iR_ir_sr_sr_ur_u$)
↓
Single-cross hybrid ♀
($R_ir_iR_sr_sr_ur_u$)

×

*D. uncinatum* ♀ × *D. intortum* ♂
($r_ir_ir_sr_sR_uR_u$) ($R_iR_ir_sr_sr_ur_u$)
↓
Single-cross hybrid ♂
($R_ir_ir_sr_sR_ur_u$)

↓
Double-cross hybrid
(Genotypes are presented in Table 5)

TABLE 5

MALE AND FEMALE GAMETES AND GENOTYPES OF THE INTERSPECIFIC CROSSES IN *Desmodium*

| FEMALE GAMETES | MALE GAMETES $R_ir_sR_u$ | $r_ir_sR_u$ | $R_ir_sr_u$ | $r_ir_sr_u$ |
|---|---|---|---|---|
| $R_iR_sr_u$ | $R_iR_iR_sr_sR_ur_u$* | $R_ir_iR_sr_sR_ur_u$* | $R_iR_iR_sr_sr_ur_u$ | $R_ir_iR_sr_sr_ur_u$ |
| $r_iR_sr_u$ | $R_ir_iR_sr_sR_ur_u$* | $r_ir_iR_sr_sR_ur_u$ | $R_ir_iR_sr_sr_ur_u$ | $r_ir_iR_sr_sr_ur_u$ |
| $R_ir_sr_u$ | $R_iR_ir_sr_sR_ur_u$ | $R_ir_ir_sr_sR_ur_u$ | $R_iR_ir_sr_sr_ur_u$ | $R_ir_ir_sr_sr_ur_u$ |
| $r_ir_sr_u$ | $R_ir_ir_sr_sR_ur_u$ | $r_ir_ir_sr_sR_ur_u$ | $R_ir_ir_sr_sr_ur_u$ | $r_ir_ir_sr_sr_ur_u$ |

*Double-cross hybrids with three dominant genes, $R_i$, $R_s$, and $R_u$.

The two single-cross hybrids had flat leaves, and were heterozygous at two loci. Each of them produced 4 types of gametes which form 16 combinations (genotypes) in double-cross hybrids, as outlined in Table 5. According to the table, 3 out of 16 double-cross hybrid plants would have rugose leaves. Chow (1968) found 4 out of 19 double-cross hybrid plants producing rugose leaves. These results, 4:15 for rugose to flat, respectively, were very close to the expected ratio, 3:13 (chi-square = 0.07, df = 1, $p > 0.75$). This indicates that the three-gene complementary action took place in the interspecific hybridization program of *Desmodium*, although the author was not aware of it.

## LITERATURE CITED

BRYAN, W. W. 1966. The pasture value of species of *Desmodium*. Internatl. Grassl. Congr. Proc. 10:311–315.

———. 1969. *Desmodium intortum* and *Desmodium uncinatum*. Herbage Abstr. 39: 183–191.

CHOW, K. H. 1968. Interspecific hybridization in the genus *Desmodium*. M.S. Thesis. University of Hawaii, Honolulu. 124 pp.

———. 1971. Morphological variation and breeding behavior of some tropical and subtropical legumes. Ph.D. Thesis. Cornell University, Ithaca, N.Y. 329 pp.

———. 1974. Morphology and ecology of some introduced herbaceous legumes. Garden's Bull. 27:85–94.

———. 1975. A dichotomous key to the *Desmodium* species based upon esterase patterns. J. Sing. Natl. Acad. Sci. 4:121–124.

CHOW, K. H., and L. V. CROWDER. 1973. Hybridization of *Desmodium* species. Euphytica 22:399–404.

———. 1974*a*. Flowering behavior and seed development in four *Desmodium* species. Agron. J. 66:236–238.

———. 1974*b*. Putative parents of a *Desmodium* selection examined morphologically and by isozyme patterns. Bot. Gaz. 135: 180–184.

———. 1975. Esterase isozyme patterns of some tropical and subtropical herbaceous legumes. Pac. Sci. 29:365–369.

HUTTON, E. M. 1960. Flowering and pollination in *Indigofera spicata*, *Phaseolus lathyroides*, *Desmodium uncinatum* and other tropical pasture legumes. Emp. J. Exp. Agric. 28:235–243.

———. 1968. Australia's pasture legumes. J. Austral. Inst. Agric. Sci. 34:203–218.

HUTTON, E. M., and S. G. GRAY. 1967. Hybridization between the legumes *Desmodium intortum*, *D. uncinatum* and *D. sandwicense*. J. Austral. Inst. Agric. Sci. 33:122–123.

MCWHIRTER, K. S. 1969. Cytoplasmic male sterility in *Desmodium*. Austral. J. Agric. Res. 20:227–241.

OHASHI, H. 1973. The genus *Desmodium* and its allied genera. Ginkgoana 1.

PRITCHARD, A. J., and K. F. GOULD. 1964. Chromosome numbers in some introduced and indigenous legumes and grasses. CSIRO Austral. Div. Trop. Past. Techn. Pap. No. 2. 18 pp.

ROTAR, P. P., and K. H. CHOW. 1971. Morphological variation and interspecific hybridization among *Desmodium intortum*, *D. sandwicense* and *D. uncinatum*. Hawaii Agric. Exp. Sta. Techn. Bull. No. 82. 28 pp.

ROTAR, P. P., and W. URATA. 1967. Cytological studies in the genus *Desmodium*; some chromosome counts. Amer. J. Bot. 54:1–4.

TAKAHASHI, M. 1952. Tropical forage legumes and browse plants research in Hawaii. Internatl. Grassl. Congr. Proc. 6:1411–1417.

———. 1956. Grassland in tropical humid lowlands of Hawaii. Internatl. Grassl. Congr. Proc. 7:547–555.

WONG, C. C. 1961. Growth, flowering and forage production of some grasses and legumes in response to different photoperiods. J. Agric. Assoc. China 36:27–52.

YOUNGE, O. R., D. L. PLUCKNETT, and P. P. ROTAR. 1964. Culture and yield performance of *Desmodium intortum* and *D. canum* in Hawaii. Hawaii Agric. Exp. Sta. Techn. Bull. No. 59. 28 pp.